

On page 46, line 27, please replace with:

---5'-CTAGTTGCAGTAGTTCTCCAGC-3' (SEQ. ID. NO. 4)---

On page 9, lines 7 to 8, please replace with:

---FIG. 14 are nucleotide sequences of rat GIP (SEQ. ID. NO. 18) and mouse chromagranin A gene promoter regions (SEQ. ID. NO. 5).---

On page 9, lines 10 to 11, please replace with:

---FIG. 15 are nucleotide sequences of promoter and exon 1 of mouse secretogranin II (Accession no. AF037451) (SEQ. ID. NO. 6) and a 5' portion of mouse glucokinase gene promoter (Accession no. U93275) (SEQ. ID. NO. 7).---

On page 9, lines 12 to 15, please replace with:

---FIG. 16 are nucleotide sequences of a 3' portion of mouse glucokinase gene promoter (Accession no. U93275), human adenosine deaminase gene promoter region (Accession no. X02189) (SEQ. ID. NO. 8); and human pre-proinsulin amino acid sequence (SEQ. ID. NO. 9), and 60 bp of a 5' region of pre-proinsulin (SEQ. ID. NO. 10).---

On page 9, lines 16 to 17, please replace with:

---FIG. 17 are nucleotide sequences of the remaining 3' portion of human pre-proinsulin (SEQ. ID. NO. 12) and a 5' portion of the human leptin gene cDNA (SEQ. ID. NO. 11).---

On page 9, lines 18 to 19, please replace with:

---FIG. 18 are nucleotide sequences of the remaining 3' portion of human leptin (SEQ. ID. NO. 14), human CCK amino acid (SEQ. ID. NO. 13) and nucleotide sequences and 60 bp of rat CCK promoter (SEQ. ID. NO. 15).---



EXAMPLE II

This example describes transgenic mice that produce insulin in response to glucose.

Using the human insulin expression construct GIP/Ins described in Example I, the 5 GIP/insulin fragment (~4.1 Kb) was removed by digestion with HindIII. Transgenic mice were generated by pronuclear microinjection of the ~4.1 Kb transgene into fertilized embryos that were implanted into pseudopregnant females. Transgenic offspring were identified by Southern blot analysis. DNA from ear sections was digested with Xhol and Pvull (FIG 3), electrophoretically separated, and transferred to nylon membrane. For the 10 detection of the transgene, a 416 bp human insulin gene fragment encompassing intron 2 was amplified using primers 2 and 4 (FIG 3). The PCR product was prepared as a probe by random labeling with $[\alpha-^{32}P]$ dCTP, and bands were detected by autoradiography. Southern analysis results were further confirmed by PCR amplification of the genomic 15 DNA using primers 2 and 4. Positive founders were outbred with wild-type FVB/N mice to establish transgenic lines (FIG 8).

Transgenic mice tissues were examined for insulin expression. In brief, total RNA (50 μ g) for each mouse stomach and duodenum, ileum, muscle, liver, spleen, kidney, fat, brain, lung, heart, bladder and testes were fractionated, transferred to a membrane and probed with a 333 base pair cDNA fragment encompassing exons 1 and 2 and part of exon 20 3 of human preproinsulin gene. The analysis revealed that insulin was expressed in the stomach and duodenum, but not in ileum, muscle, liver, spleen, kidney, fat, brain, lung, heart, bladder or testes from the resulting transgenic animals (FIG 9).

To confirm insulin production in duodenum, RT-PCR analysis for insulin mRNA was performed. In brief, human proinsulin specific, forward 5'-
25 CCAGCCGCAGCCTTGTA-3' and reverse 5'-GGTACAGCATTGTTCCACAATG-
3' mouse proinsulin specific, forward 5'-ACCACCAGCCCTAAGTGAT-3' and reverse
5'-CTAGTTGCAGTAGTTCTCCAGC-3' primers used were. PCR conditions were as
follows: denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C
for 1 min for 45 cycles. PCR products were analyzed on a 2% agarose gel and visualized

FIG. 12 is a graph showing the changes of blood glucose concentration in normal control mice, streptozotocin (STZ)-treated control mice and STZ-treated transgenic mice following an oral glucose challenge (1.5 g glucose / kg body weight).

FIG. 13 is a series of micrographs showing immunohistochemical staining for 5 mouse insulin in pancreatic sections from control and STZ-treated transgenic mice. Arrows indicate islets.

FIG. 14 are nucleotide sequences of rat GIP and mouse chromagranin A gene promoter regions. (SEQ ID NO 18) (SEQ ID NO 5)

FIG. 15 are nucleotide sequences of promoter and exon 1 of mouse secretogranin 10 II (Accession no. AF037451) and a 5' portion of mouse glucokinase gene promoter (Accession no. U93275). (SEQ ID NO 6) (SEQ ID NO 7)

FIG. 16 are nucleotide sequences of a 3' portion of mouse glucokinase gene promoter (Accession no. U93275), human adenosine deaminase gene promoter region (Accession no. X02189); and human pre-proinsulin amino acid sequence, and 60 bp of a 15 5' region of pre-proinsulin. (SEQ ID NO 8) (SEQ ID NO 9) (SEQ ID NO 10) (SEQ ID NO 11)

FIG. 17 are nucleotide sequences of the remaining 3' portion of human pre-proinsulin and a 5' portion of the human leptin gene cDNA. (SEQ ID NO 12) (SEQ ID NO 11) (SEQ ID NO 13) (SEQ ID NO 14)

FIG. 18 are nucleotide sequences of the remaining 3' portion of human leptin, human CCK amino acid and nucleotide sequences and 60 bp of rat CCK promoter. (SEQ ID NO 15) (SEQ ID NO 16) (SEQ ID NO 17) (SEQ ID NO 18)

FIG. 19 are nucleotide sequences of the remaining 3' portion of rat CCK promoter and amino acid and nucleotide sequences of human growth hormone. (SEQ ID NO 19)

DETAILED DESCRIPTION

The invention is based, in part, on the targeted production of a protein in a tissue of 25 animals at levels sufficient to provide therapy. More specifically, the invention includes methods of targeting expression of any protein of interest to endocrine cells in the gastrointestinal tract of a subject such that the protein is released into the bloodstream of the subject in a regulated manner. Genetic constructs including an expression control element (e.g., promoter) that targets gene expression to gut endocrine cells operably linked